MALARIA BLOOD STAGE PARASITES ACTIVATE HUMAN PLASMACYTOID DENDRITIC CELLS AND MURINE DENDRITIC CELLS THROUGH A TOLL-LIKE RECEPTOR 9-DEPENDENT PATHWAY

Pichyangkul S, Yongvanitchit K, Kum-arb U, Hemmi H, Akira S, Krieg AM, Heppner DG, Stewart VA, Hasegawa H, Looareesuwan S, Shanks GD and Miller RS

A common feature of severe *Plasmodium falciparum* infection is the increased systemic release of proinflammatory cytokines that contributes to the pathogenesis of malaria. Using human blood, we found that blood stage schizonts or soluble schizont extracts activated plasmacytoid dendritic cells (PDCs) to up-regulate CD86 expression and produce IFN-α. IFN-α production was also detected in malaria-infected patients, but the levels of circulating PDCs were markedly reduced, possibly because of schizont-stimulated up-regulation of CCR7, which is critical for PDC migration. The schizont-stimulated PDCs elicited a poor T cell response, but promoted γδ T cell proliferation and IFN-γ production. The schizont immune stimulatory effects could be reproduced using murine DCs and required the Toll-like receptor 9 (TLR9)-MyD88 signaling pathway. Although the only known TLR9 ligand is CpG motifs in pathogen DNA, the activity of the soluble schizont extract was far greater than that of schizont DNA, and it was heat labile and precipitable with ammonium sulfate, unlike the activity of bacterial DNA. These results demonstrate that schizont extracts contain a novel and previously unknown ligand for TLR9 and suggest that the stimulatory effects of this ligand on PDCs may play a key role in immuno-regulation and immunopathogenesis of human falciparum malaria.

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MALARIA BLOOD STAGE PARASITES ACTIVATE HUMAN PLASMACYTOID DENDRITIC CELLS AND MURINE DENDRITIC CELLS THROUGH A TLR9-DEPENDENT PATHWAY

Pichyangkul S, Youngvanitchit K, Kum-arb U, Mahanonda R and Miller RS

A common feature of severe *P. falciparum* infection is the increased systemic release of pro-inflammatory cytokines that contributes to the pathogenesis of malaria. Using human blood, we found that blood stage schizonts or soluble schizont extracts activated plasmacytoid dendritic cells (PDCs) to up-regulate CD86 expression and produce IFN-a. The IFN-a production was also detected in malaria-infected patients, but the levels of circulating PDCs were markedly reduced, possibly because of schizont-stimulated up-regulation of CC chemokine receptor 7 (CCR7), which is critical for PDC migration. The schizont-stimulated PDCs elicited a poor T cell response, but promoted gd T cell proliferation and IFN-g production. The schizont immune stimulatory effects could be reproduced using murine DCs, and required the Toll-like receptor 9 (TLR9)-MyD88 signaling pathway. Although the only known TLR9 ligand is CpG motifs in pathogen DNA, the activity of the soluble schizont extract was far higher than that of schizont DNA, and the activity was heat labile and precipitable with ammonium sulfate, unlike the

activity of bacterial DNA. These results demonstrate that schizont extracts contain a novel and previously unknown ligand for TLR9, and suggest that the stimulatory effects of this ligand on PDCs may play a key role in immunoregulation and immunopathogenesis of human falciparum malaria.

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MEASURING ALLELIC HETEROGENEITY IN *PLASMODIUM FALCIPARUM* BY HETERODUPLEX TRACKING ASSAY

Ngrenngarmlert W, Kwiek J, Kamwendo D, Ritola K, Swanstrom R, Wongsrichanalai C, Ittarat W and Meshnick S

We developed a novel *Plasmodium falciparum* enotyping strategy based on the heteroduplex tracking assay (HTA) method commonly used to genotype viruses. Because it can detect both sequence and size polymorphisms, we hypothesized that HTA is more sensitive than current methods. To test this hypothesis, we compared the ability of HTA and nested PCR to detect genetic diversity in seventeen Thai samples; although nested PCR detected more variants in 2/17 cases, HTA identified more *P. falciparum* strains in 9/17 cases, suggesting that HTA is equal to if not more sensitive than nested PCR. Furthermore, HTA differentiated between re-infection and recrudescence in seven paired admission and recurrent patient samples. This study is a proof of concept that HTA is a sensitive allelic discrimination method able to determine genetic diversity in *P. falciparum* and warrants its use in studies of antimalarial drug efficacy.

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A NEW METHOD FOR DETECTION OF *PFMDR1* MUTATIONS IN *PLASMODIUM FALCIPARUM* DNA USING REAL TIME PCR

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Background: Surveillance for drug-resistant *Plasmodium falciparum* should be a component of malaria control programmes. Real-time PCR methods for the detection of parasite single-nucleotide polymorphisms (SNPs) and gene amplification could be useful surveillance tools.

Methods: A real-time PCR assay has been developed that identifies single nucleotide polymorphisms (SNPs) at amino acids 86, 184, 1034 and 1042 in the *P. falciparum* multi-drug resistant (*pfmdr1*) gene that may be associated with anti-malarial drug resistance.